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BIOCHEMISTRY OF SULFUR-CONTAINING AMINO ACIDS

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CONTENTS

PERSPECTIVES AND SUMMARY

The literature on sulfur amino acid metabolism is too vast for a short chapter to cover in great depth. I attempt here a brief overview with references to many specialized review articles. This review emphasizes aspects of sulfur amino acid metabolism elucidated in the last ten years, in

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particular aspects not generally covered in biochemistry texts, e.g. transaminative pathways of methionine metabolism. A selected list of reviews is given in references 1–15. References to reviews on glutathione are covered in the chapter by A. Meister in this volume (1a).

THE SULFUR CYCLE

Most biochemists are familiar with the outlines of the carbon, nitrogen, and sulfur cycles. Although the kinetics are not fully understood, there is no doubt that sulfur is recycled through the biosphere in considerable amounts (16–18). As with other cycles, a variety of organisms plays a role in both assimilation and dissimilation reactions (Figure 1). Mammals dissimilate sulfur by breakdown of sulfur amino acids, but cannot assimilate, i.e. meet their requirements with, inorganic sulfur, and must instead rely principally on ingested methionine and cysteine.

ASSIMILATION OF SULFUR INTO AMINO ACIDS IN MICROORGANISMS

Reductive dissimilation of sulfate occurs in certain obligatory anaerobic bacteria, such as *Desulfovibrio* and *Desulfotomaculum*. This reduction pathway produces hydrogen sulfide that is largely lost to the environment. The sulfate dissimilatory bacteria oxidize organic compounds or

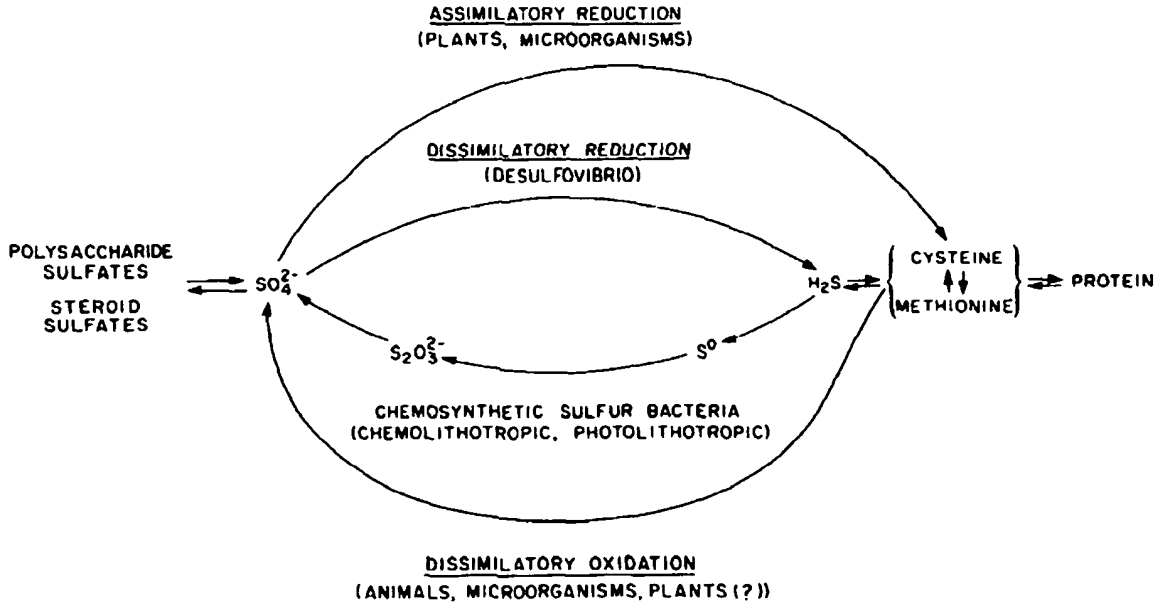
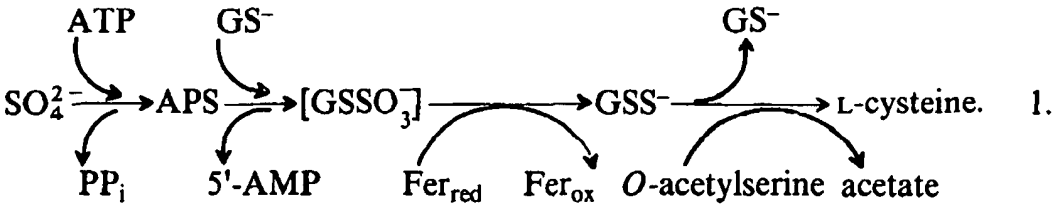


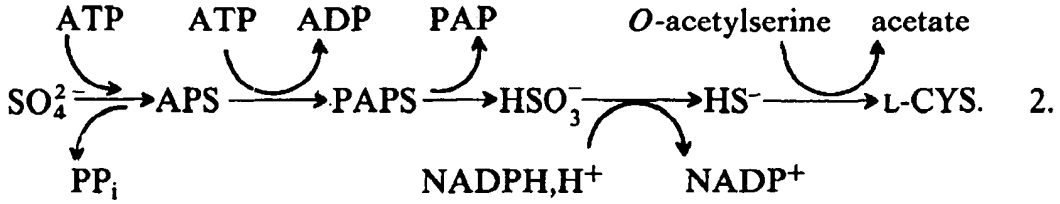
Figure 1 Schematic representation of flow of sulfur through the biosphere, modified from (16, 18).

molecular hydrogen by using metabolites of sulfate as a terminal electron acceptor in a manner similar to the way aerobes utilize oxygen as an acceptor (6, 16). Reduction of sulfate can also result in assimilation rather than loss of the reduced form of sulfur (Figure 1). Plants and many microorganisms, but not higher animals, have evolved mechanisms for sulfate assimilation. Reductive assimilation of sulfate, i.e. incorporation of sulfate sulfur into thiol groups of amino acids and other bioorganic compounds, occurs via two major enzymatic routes: the APS (adenosine 5'-phosphosulfate) pathway and the PAPS (adenosine 3'-phosphate-5'-phosphosulfate) pathway. In the APS assimilation pathway (Equation 1), which requires ferredoxin, glutathione (GSH) reacts with APS to yield the thiosulfate, $GSSO_3^-$ (a Bunte salt), which is converted in turn to GSS^- via a thiosulfate reductive reaction; GSS^- reacts with *O*-acetylserine to yield cysteine. In some organisms, other -SH compounds can partially replace GSH as a thiol carrier (18):



1.

In the PAPS pathway, *O*-acetylserine reacts directly with HS^- (Equation 2):



2.

In yeast, conversion of PAPS to sulfite requires thioredoxin, but the exact role of thioredoxin is not yet fully elucidated (18). It was originally thought that thioredoxin was also a cofactor in the PAPS pathway of *Escherichia coli,* but in *E. coli* mutants lacking this protein, glutaredoxin can substitute (19). The APS pathway has been found in *Chlorella, Euglena,* spinach chloroplasts, and *Lemna* (duckweed) (e.g. 19–23). The PAPS system has been studied most extensively in yeast (24), *E. coli* (25), and *Salmonella typhimurium* (26).

Alternative routes for assimilation of sulfide are also known (Equations 3–5):

O-Acetylhomoserine + H_2S → L-homocysteine + acetate. 3.

O-Succinylhomoserine + H_2S → L-homocysteine + succinate. 4.

O-Phosphohomoserine + H_2S → L-homocysteine + P_i. 5.

O-Acetylhomoserine is the preferred substrate in spinach (27) and fungi (28). *O*-Succinylhomoserine is the preferred substrate in *E. coli* (28). *Chlorella* and *Lemnis* preferentially utilize *O*-phosphohomoserine, although *Chlorella* uses the *O*-malonyl-, *O*-oxalyl- and *O*-succinylhomoserine derivatives as well (29).

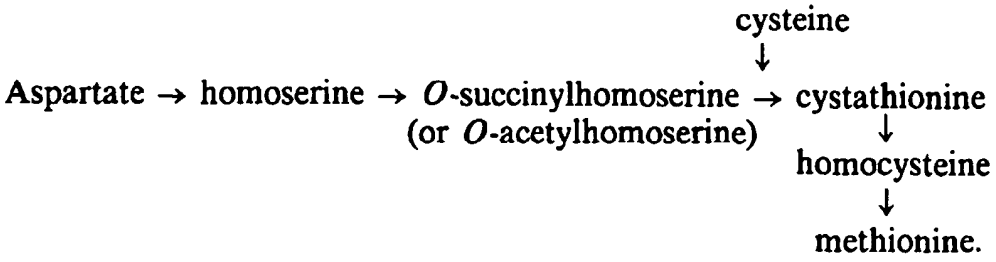
In 1957, Schlossmann & Lynen (30) showed that yeast contains an enzyme that catalyzes the reversible conversion of L-serine to L-cysteine (Equation 6):

L-serine + $H_2S \leftrightharpoons$ L-cysteine + H_2O. 6.

Serine sulfhydrase (cysteine synthase) was found subsequently to be widely distributed in nature, including mammalian tissues, and to have a very wide specificity (cf 31). In fact, there are similarities between cysteine synthase and cystathione β-synthase (32, 33); because (*a*) vertebrates do not possess a sulfate-reducing system, (*b*) in vivo incorporation of sulfide (S^{2-}) into cysteine is of minor importance, and (*c*) equilibrium lies far toward serine formation (34), the function of the enzyme in vertebrates is probably to synthesize cystathionine. Thus, cysteine synthase may be regarded as a variant cystathionine β-synthase; both enzymes are "C_3-specific β-replacing lyases" (33).

METHIONINE FORMATION IN MICROORGANISMS

In microorganisms, the major route to methionine is via cysteine and homocysteine (Equation 7). In vertebrates the reverse is true, i.e. methionine serves as a precursor of homocysteine and cysteine sulfur. A pivotal difference in the two groups is the fate of cystathionine. In microorganisms, β-cystathionase splits a C–S bond resulting in formation of homocysteine (Equation 7). In mammals, γ-cystathionase splits the other C–S bond resulting in formation of cysteine.



7.

Flavin (35) and Woods et al (36) have presented detailed reviews of methionine biosynthesis in microorganisms. A few points are discussed here. In *E. coli, S. typhimurium,* and other enteric bacteria, the immediate precursor of cystathionine is *O*-succinylhomoserine (37). In *Neurospora crassa* (38) and *Aspergillus nidulans* (38) the immediate precursor is *O*-acetylhomoserine. Methylation of homocysteine in microorganisms can occur via two distinct pathways. Equation 8 shows the vitamin B_{12}-dependent homocysteine transmethylation reaction. This reaction requires a reducing system and can also utilize S-adenosylmethionine in place of methyltetrahydrofolate as methyl donor; 2-mercaptoethanol can act as a methyl acceptor, although less readily than homocysteine (39). Equation 9 shows the vitamin B_{12}-independent reaction. This reaction requires P_i and is stimulated by Mg^{2+}.

$$5\text{-}CH_3\text{-}H_4PteGLU + \text{L-homocysteine} \xrightarrow{B_{12}} \text{L-methionine} + H_4PteGLU. \quad 8.$$

$$5\text{-}CH_3\text{-}H_4PteGLU_{3\text{-}7} + \text{L-homocysteine} \xrightarrow{P_i,\ Mg^{2+}} \text{L-methionine} + H_4PteGLU_{3\text{-}7}. \quad 9.$$

Many microorganisms and plants can synthesize homocysteine both by direct incorporation of H_2S (Equations 3–5) or by the cystathionine pathway (Equation 7). There has been some debate as to which is the most important route to methionine in these organisms (cf 35, 40). In plants (40) and in *A. nidulans* (41) the transsulfuration pathway probably predominates. On the other hand, recent evidence suggests that the major route for homocysteine synthesis in *Brevibacterium flavum* is via reaction of H_2S with *O*-acetylhomoserine (Equation 3; 42). Detailed reviews of methionine metabolism in plants and microorganisms have recently been published (43, 44).

TRANSSULFURATION PATHWAY OF METHIONINE DEGRADATION

Before 1940, cyst(e)ine was regarded as an essential dietary amino acid in mammals; later it was concluded that methionine rather than cysteine is an essential amino acid, and that cysteine sulfur could be derived from methionine (45). However, in the presence of adequate methyl donors, the methionine requirement may be met solely from ingested homocysteine (46). Moreover, it has recently been found in several species that cyst(e)ine is not indispensable after all. For example, it can supply 50% of the total dietary sulfur amino acid requirement in growing beagle dogs (47). Sturman et al

were unable to detect cystathionase activity in the livers of human fetuses (48) and concluded that cyst(e)ine is indeed an essential amino acid for the human fetus (48, 49).

The transsulfuration pathway (Figure 2) is now well characterized (e.g. 2, 8, 12, 31, 46) so that only a few points are discussed here. Figure 2 shows that methionine and homocysteine are readily interconvertible. This has led some authors to use the term "methionine cycle." Mudd (46) concluded that, via this cycle, "methionine is controlled to just the right level for transmethylation reactions and polyamine synthesis." Evidently, much of the homocysteine skeleton is efficiently re-utilized. Mudd & Pool (50) calculated that the methyl group is conserved for an average of 1.9 turnovers in men, 1.5 in women. Krebs et al (51) investigated methionine metabolism and concluded that levels are controlled to a fine degree at the level of homocysteine: when methionine is needed, homocysteine is re-methylated by methyltetrahydrofolate; when methionine is in excess, catabolism of homocysteine via the γ-cystathionase reaction is accelerated.

Figure 2 shows two salvage pathways for methionine, methionine synthase and betaine-homocysteine methyltransferase. Some texts also state that dimethylthetin and dimethyl-β-propiothetin (cf 52) are methyl donors,

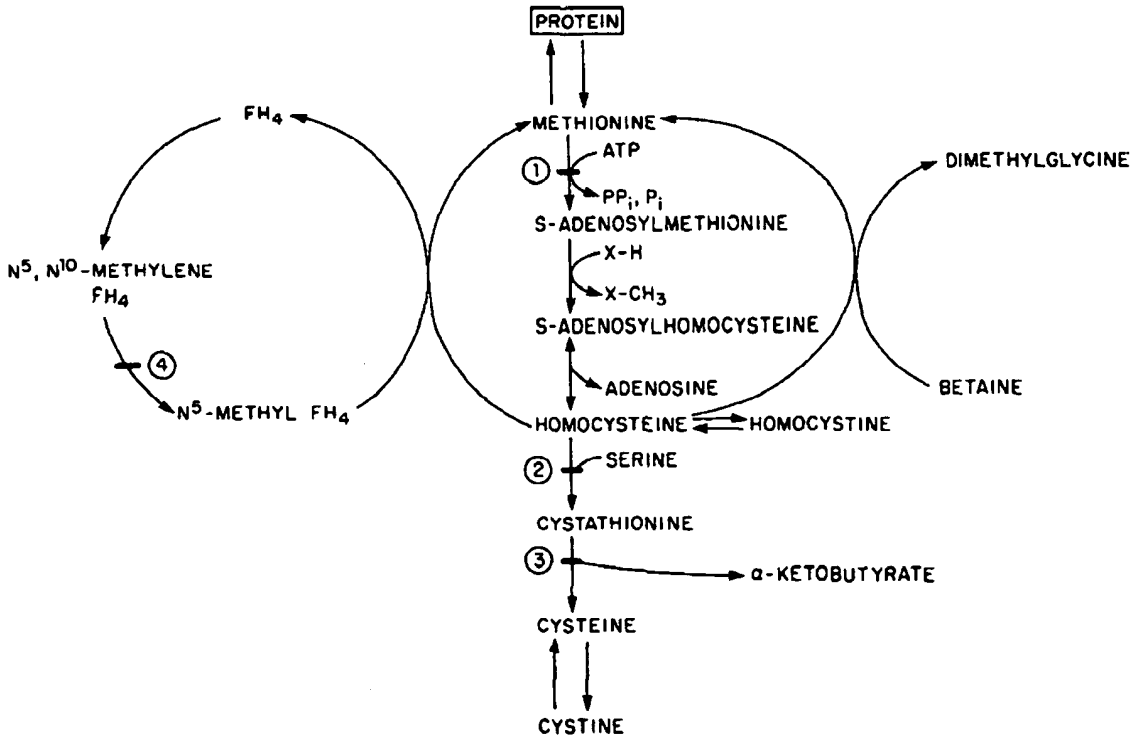


Figure 2 Transsulfuration pathway to cysteine. The solid bars and numbers indicate enzymes known to be absent or modified in various metabolic defects of man.

but the significance of this reaction is not clear. A third pathway (salvage from 5'-methylthioadenosine) is discussed later. Some authors include a desulfhydrase reaction (Equation 10) as a route for metabolism of homocysteine. However, the enzyme responsible, homocysteine desulfhydrase, is identical to γ-cystathionase (53), and the reaction is probably of little or no significance in mammalian tissues.

$$\text{L-Homocysteine} + H_2O \rightarrow \alpha\text{-ketobutyrate} + NH_3 + H_2S. \quad 10.$$

TRANSAMINATIVE PATHWAY OF METHIONINE CATABOLISM

Large amounts of methionine are toxic, whether derived from the diet (e.g. 54–56) or from metabolic blocks in diseases such as liver dysfunction (57, 58) and some forms of hypermethionemia (e.g. 59). In patients with liver disease the transsulfuration pathway is diminished (60) leading to elevated mercaptans in blood (61) and in the breath (58, 61, 62). Benevenga (63) concluded that the metabolic basis for methionine toxicity cannot be attributed to catabolism via the transsulfuration pathway but is related to metabolism of the methyl moiety. Subsequently, Case & Benevenga (64, 65) showed that methionine can be extensively catabolized by a pathway independent of S-adenosylmethionine formation and that formaldehyde and formate were two intermediates in the oxidation of the methyl carbon by this pathway. From detailed experiments with labeled methionine, Benevenga and colleagues suggested a "transaminative" pathway of methionine catabolism in which methanethiol is a breakdown product (65–71; Figure 3).[1] Canellakis & Tarver (74) first showed that α-keto-γ-methiolbutyrate is a better precursor of methanethiol than is methionine.

A decrease in the transsulfuration pathway relative to the transamination pathway may explain the increase in methyl mercaptan and dimethyl disulfide in patients with liver disease. Moreover, the similarity in tissue damage brought about by both excess methionine and by 3-methylthiopropionate suggests that methionine toxicity is related to methanethiol and H_2S, both of which are extremely poisonous (69, 70). Steele & Benevenga (69) pointed out several lines of evidence indicating that the transaminative pathway is operative at physiological levels of methionine intake. Dimethyldisulfide is an attractant pheromone in hamster vaginal secretions (75). Low levels of methanethiol and dimethylsulfide can be detected in the

[1]Steele and Benevenga have also shown that ethionine is metabolized, at least in part, via a transaminative pathway in rat liver (72). This finding may explain some of the toxicity associated with ethionine; a major metabolite of this pathway is the markedly toxic 3-ethylthiopropionate (73).

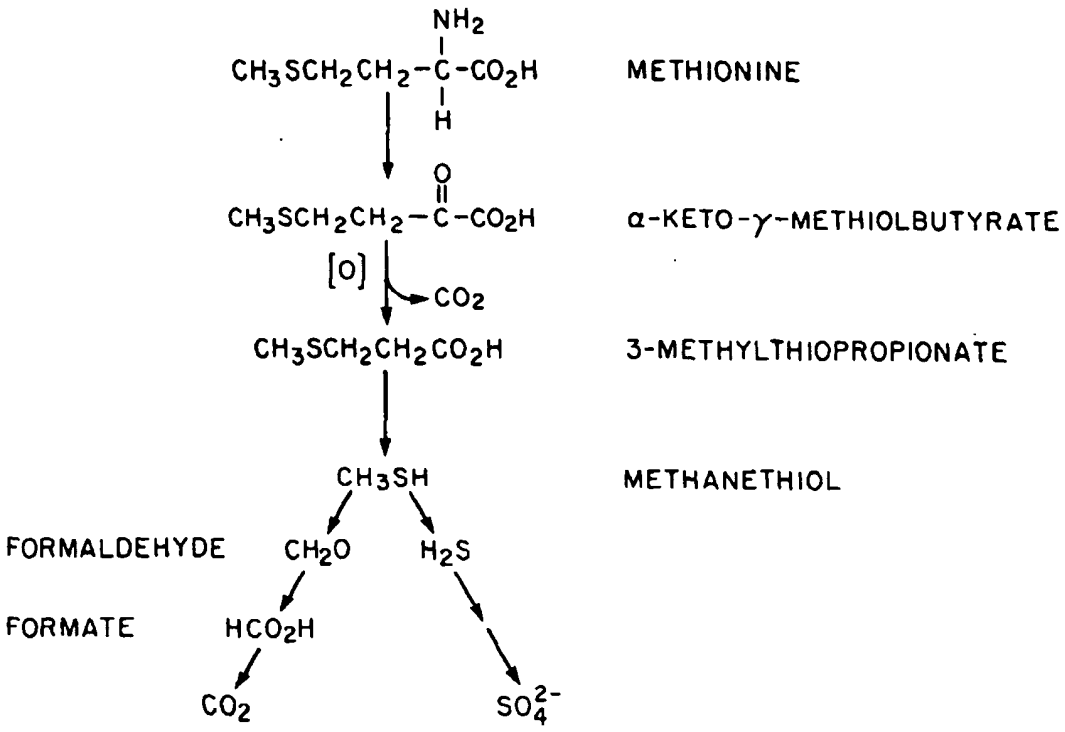


Figure 3 Transaminative pathway of methionine metabolism, modified from (65, 67).

breath of normal individuals (62). Toohey (76) discovered a thioalkane, tentatively identified as methanethiol, that is required for cell division in certain cell lines.

Rat tissue preparations can catalyze transamination between methionine and α-ketoglutarate (77), and methionine is a substrate of purified preparations of rat liver glutamine transaminase (78), rat kidney glutamine transaminase (79), and rat liver asparagine transaminase (80). Ikeda et al (81) demonstrated that a leucine transaminase isolated from rat liver mitochondria can transaminate methionine. However, the transaminase(s) responsible for the formation of α-keto-γ-methiolbutyrate in vivo remain unknown. The glutamine transaminases are probably not responsible. The much higher levels of glutamine in rat tissues, coupled with the virtually irreversible nature of the reaction with glutamine should ensure that the reaction is directed toward glutamine utilization (82, 83). Furthermore, pyruvate and especially α-ketoglutarate are poor substrates of the enzymes. The glutamine transaminases may act to spare the carbon skeleton of α-keto-γ-methiolbutyrate, arising from nonspecific transamination of methionine, at the expense of "nonessential" glutamine; without such a mechanism an excessive loss of essential methionine, via degradation of α-keto-γ-methiolbutyrate, might occur (78, 79, 82, 83). Recent evidence suggests that α-keto-γ-methiolbutyrate is oxidatively decarboxylated by branch-chain α-keto acid dehydrogenase (84). The presence of trace

amounts of β-methylmercaptopropionaldehyde (methional) in foodstuffs (85) suggests that nonoxidative decarboxylation of α-keto-γ-methiolbutyrate may also occur. Evidently, salvage relative to degradation depends on availability of glutamine and competition from endogenous α-keto acids. Interestingly, glutamine is rapidly metabolized by rat hepatocytes in the presence of α-keto-γ-methiolbutyrate but not in the presence of pyruvate (86). Moreover, very recent evidence suggests that the glutamine transaminase reaction acts to salvage α-keto-γ-methiolbutyrate formed from 5'-methylthioadenosine (see below).

Using a gas chromatographic–mass spectrometric determination of the quinoxalinol derivative, Kaji et al (87) showed that α-keto-γ-methiolbutyrate occurs in trace amounts in normal urine. The urinary output of the α-keto acid increased after oral loading with D- or L-methionine. Kaji et al (62, 87) also showed that exhalation of dimethylsulfide is much greater following oral loading of D-methionine than with L-methionine. The authors suggested that the mechanism of Benevenga is more important for the metabolism of D-methionine than for L-methionine (87). [Presumably, α-keto-γ-methiolbutyrate is formed from D-methionine by the action of D-amino acid oxidase; D-methionine is an excellent substrate of this enzyme (88)]. Hydrogen sulfide, one of the proposed intermediates of the transaminative pathway, may also arise enzymatically from cyst(e)ine or from 3-mercaptopyruvate (see below). Although not detected in mammalian tissues, H_2S is present in trace amounts in flatus (89). The relative importance of the transaminative pathway vs the transsulfuration pathway of L-methionine breakdown must await further study. Careful methyl balance studies by Mudd and colleagues (e.g. 46) suggest that the transsulfuration pathway greatly predominates. However, impairment of the transsulfuration pathway, as in liver disease (57, 58) and/or in portacaval shunting (90), probably leads to a relative increase in the transaminative pathway.

S-ADENOSYLMETHIONINE METABOLISM

General Considerations

Following (*a*) the realization of the biological importance of 5'-methylthioadenosine (MTA) in 1952 (91) and (*b*) the discovery of S-adenosylmethionine (SAM) in 1953 (92), Tabor et al showed that a number of microorganisms utilize SAM to convert putrescine to spermine and to spermidine (93). At the same time, Shapiro & Mather (94) showed that SAM could be rapidly degraded to MTA (and homoserine lactone) and then to 5-methylthioribose (MTR) by *Aerobacter aerogenes*. Later, Pegg & Williams-Ashman (95) showed that the rat ventral prostate has an enzyme system that catalyzes the SAM-mediated conversion of putrescine to spermidine.

Figure 4 gives a scheme of some of the known metabolic routes of SAM in prokaryotes (cf 96). Cleavage reactions involving all three of the S–C bonds are known. By far the most numerous are those reactions in which cleavage results in methylation (Figure 4; 12). Although methylation of relatively small molecules has long been known, the importance of SAM in the methylation of phospholipids (97), proteins (98), polysaccharides (99), and nucleic acids (100) is becoming increasingly apparent. In a few cases, S–C cleavage results in transfer of the adenosyl portion to enzyme protein (e.g. 101). Finally, breakage of a S–C bond with transfer of the 3-amino-3-carboxypropyl group to tRNA also occurs (102). Hydrolysis of SAM to MTA and homoserine lactone (Figure 4) is a special case of cleavage of this third S-C bond.

Salvage of Methionine from 5'-Methylthioadenosine and α-Keto-γ-Methiolbutyrate

Prokaryotes generally convert MTA to MTR and adenosine. However, mammalian cells convert MTA to 5'-methylthioribose 1-phosphate (MTRP) and adenine in a reaction catalyzed by 5'-methylthioadenosine phosphorylase (103, 104). The enzyme is present in a number of rat organs (104, 105) and in human prostate (106) and placenta (107). A single example of the enzyme occurring in a prokaryote has been recorded, i.e. in the extreme thermophile *Caldariella acidophila* grown optimally at 87°C (108). Until very recently very little was known about the metabolism of MTR and

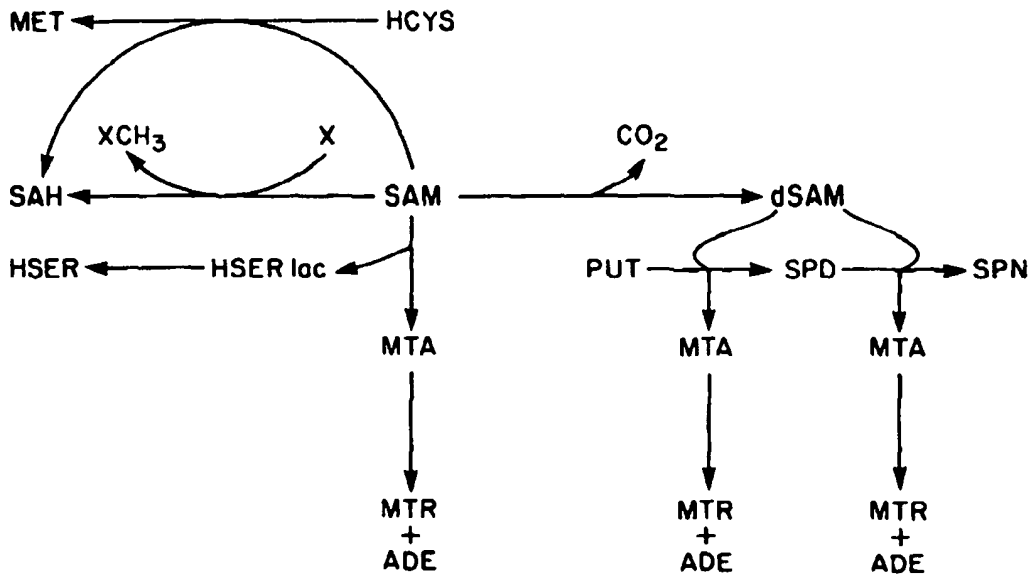


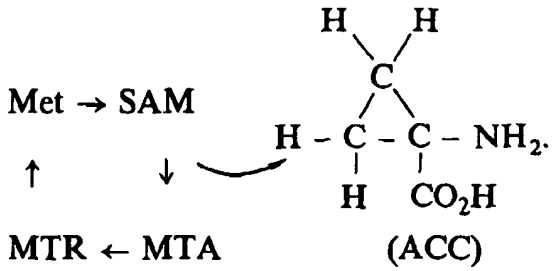
Figure 4 Some of the known metabolic routes of S-adenosylmethionine in prokaryotes. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; HCYS, homocysteine; dSAM, decarboxylated S-adenosylmethionine; SPD, spermidine; SPN, spermine; PUT, putrescine; HSER, homoserine; HSER lac, homoserine lactone; MTA, 5'-methylthioadenosine; MTR, 5-methylthioribose; ADE, adenine. X = methyl acceptor. Modified from (96).

MTRP, although in 1964 Schlenk & Ehninger (109) showed that the carbon and sulfur of the thiomethyl group of MTA is efficiently incorporated back into SAM by *Candida utilis*. Then, in 1981, Backlund & Smith (110) showed that MTA is converted to methionine by rat liver extracts. Carbons from the ribose portion, carbon and hydrogen of the methyl group, and the sulfur of MTA are all incorporated into methionine (110, 111). The authors proposed that the "pathway appears to be a significant salvage pathway for methionine synthesis in mammals, and may be necessary for removal of 5'-methylthioadenosine produced as by-product of polyamine biosynthesis." Apparently, the pathway is as follows:

$$\text{MTA} \rightarrow \text{MTRP} \rightarrow \rightarrow \rightarrow \alpha\text{KMB} \xrightarrow{\text{A.A. K.A.}} \text{MET}. \qquad 11.$$

MTRP is converted to α-keto-γ-methiolbutyrate (αKMB) by an unknown mechanism (111, 111a). α-Keto-γ-methiolbutyrate is then converted to methionine by transamination with a suitable amino acid donor (A. A., Equation 11). Glutamine and asparagine are the preferred donors. α-Keto-γ-methiolbutyrate is an excellent substrate of rat kidney and liver glutamine transaminases (78, 79) and a moderately good substrate of rat liver asparagine transaminase (80). The findings of Backlund et al (110, 111) support the earlier suggestion that one role of the glutamine transaminase is to salvage α-keto-γ-methiolbutyrate (78, 79, 82, 83).

Ethylene promotes ripening of fruit and is derived from methionine via 1-aminocyclopropane-1-carboxylate (ACC) (112). Much of the pathway in the apple has been recently elucidated (113; Equations 12, 13). Methionine is efficiently salvaged from 5'-methylthioribose (MTR) (113; Equation 12). Presumably, the mechanism is similar to that of the methionine salvage from MTRP in rat liver (110, 111) but α-keto-γ-methiolbutyrate has not yet been identified as a precursor in the apple salvage pathway:



12.

$$\text{ACC} \xrightarrow{O_2} \text{CH}_2{=}\text{CH}_2 + \text{CO}_2 + \text{HCO}_2\text{H} + \text{NH}_3. \qquad 13.$$